Microstructural and functional plasticity following repeated brain stimulation during cognitive training in non-demented older adults

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Abstract

The combination of repeated behavioral training with transcranial direct current stimulation (tDCS) holds promise to exert beneficial effects on brain function beyond the trained task. However, little is known about the underlying mechanisms. This was addressed by multimodal magnetic resonance imaging (MRI) before and after a three-week executive function training with prefrontal excitatory tDCS in 48 older adults. Results demonstrate that training combined with active tDCS enhanced prefrontal white matter microstructure which predicted individual performance gain. Training-plus-tDCS also resulted in microstructural grey matter reductions at the stimulation site, and increased prefrontal functional connectivity. We provide insight into the mechanisms underlying neuromodulatory interventions, suggesting tDCS-induced changes in fiber organization and myelin formation, glia-related and synaptic processes in the target region, and synchronization within targeted functional networks. These findings advance the mechanistic understanding of neural tDCS effects, thereby contributing to more targeted neural network modulation in future experimental and translation tDCS applications.

Introduction

Developing effective cognitive interventions to reduce or even prevent age-associated brain impairment has received substantial scientific attention in aging societies worldwide. Preliminary evidence suggests that the combination of behavioral training and concurrent transcranial electrical stimulation (tES), one of the most widely used non-invasive brain stimulation (NIBS) techniques, may induce cross-task cognitive benefits in advanced age. However, add-on effects are often small and variable. Therefore, a better understanding of the underlying mechanisms by which tDCS exerts its beneficial effects in aging brains is of utmost importance to advance the potential of this technique.

As for learning-related brain plasticity, the brain's microstructure can be modified by learning. In-vivo short-term structural plasticity, quantified by diffusion tensor imaging (DTI), has been demonstrated in white matter pathways and grey matter structures and has been linked to behavioral performance gains and neural activity alterations. DTI is considered the state-of-the-art method for studying microstructural features in the human brain. Parameters from DTI sensitive to microstructural changes are fractional anisotropy (FA) and mean diffusivity (MD) with FA in white matter pathways reflecting directional coherence of fibers and MD in grey matter reflecting magnitude of water molecule diffusion. At the cellular level, changes in neural and non-neural dependent activity (e.g., synaptogenesis and changes in dendritic spine morphology) and changes in white matter (e.g., variation of axon diameter, myelin, packing density, fiber geometry) contribute to the observed alterations in neuroimaging data. Importantly, microstructural alterations such as structural remodeling within brain tissue relevant for a given function occur within short timescales after learning. Thus, behavioral improvement is likely associated with plastic changes of brain (cellular or molecular) microstructure.
TES non-invasively modulates excitability and synaptic plasticity in neurons \(^2\) and, on the behavioral level is capable of boosting immediate performance gains and even resulting in transfer and long-term maintenance of these effects over months \(^1,12\). Repeated tDCS sessions can induce long-lasting changes in excitability and synaptic efficiency, inducing long-term potentiation (LTP)-like effects \(^13,14,15\). Simultaneous tDCS-fMRI application in proof-of-principle studies revealed changes in local activity and functional connectivity (temporally coherent activity between brain regions) that predicted behavioral performance gains \(^16,17,18\). Functional connectivity modulations in response to repeated training sessions may even reflect network level reorganizations, promoting longer-lasting neural plasticity \(^19,20,21,22\).

Establishing the underlying cellular (and molecular) mechanisms in the human brain can advance understanding of neuromodulatory plasticity. Animal models suggest modified tissue density due to altered neuronal morphology (e.g. size/shape of axons, dendritic spines and cell bodies), altered glial cells activity or reorganization/reshaping of synaptic connections as neuroplastic phenomena induced by tDCS \(^14,23\). Combined repeated tDCS-plus-training interventions in human participants which promote plasticity have been suggested to induce microstructural changes in brain white and grey matter similar to those induced by learning, but direct evidence is limited \(^15\). Importantly, multimodal imaging is necessary to establish a comprehensive understanding of the underlying neurophysiological mechanisms \(^24\).

Here, we tested the hypotheses that concurrent anodal prefrontal tDCS administered across repeated cognitive training sessions would improve white matter microstructure in cortical target areas and associated neural networks compared to training with placebo (sham) stimulation. tDCS (1 mA) was administered for 20 min concurrently with two executive function training tasks (letter updating training, decision-making). For individual fiber tractography and quantification of white matter microstructure, we used DTI acquired before and after the intervention. Further, DTI allowed us to examine whether microstructural properties in the stimulation target would change due to the intervention as suggested previously \(^22\). The investigation of microstructural plasticity markers was complemented by resting-state functional magnetic resonance imaging (rs-fMRI) to analyze functional synchrony modifications within the targeted (fronto-parietal) network. In order to explore the behavioral relevance of neural alterations, we further performed correlational analyses with N-back (transfer) task performance which was enhanced after in the target compared to the control intervention \(^25\).

**Results**

Multimodal MRI data of 48 nondemented older adults were included in a randomized, placebo-controlled study investigating potential add-on effects of tDCS during cognitive training \(^25,26\). Participants were randomly assigned to two groups (anodal and sham tDCS) using stratified blockwise randomization (based on age and baseline performance of participants). All participated in three weekly training sessions provided over three weeks (nine sessions total). The training comprised letter updating and
decision-making tasks, lasting approximately 40 min. TDCS was applied daily with an intensity of 1 mA for 20 min, starting briefly prior to the first training task. A conventional tDCS montage was used that targeted prefrontal functions \(^{27}\). The anode was centered over the left dorsolateral prefrontal cortex (F3 of the 10–20 EEG system; size: 5 cm diameter); the cathode over the contralateral supraorbital area (Fp2; size: 5 cm diameter). MRI was performed two days prior to and two days after the intervention (Table 1, Fig. 1). MRI comprised different imaging modalities to investigate effects on neural networks (Methods), such as DTI to quantify structural plasticity due to the intervention in white matter tracts using individual probabilistic tractography (defined as fractional anisotropy, FA, which reflects directional preference of diffusion) as well as in grey matter microstructure of the stimulation target (defined as mean diffusivity, MD, which reflects molecular diffusion rate) and resting-state functional resonance imaging (fMRI) to examine alterations in functional network connectivity (FC, defined as temporal correlation of blood-oxygenation level-dependent, BOLD, signals in areas of the network). Common software pipelines were used for MR data analyses. We investigated the differences of FA, MD, and FC between anodal and sham groups after the intervention to scrutinize potential add-on effects of anodal tDCS during cognitive training. We also explored linear relationships between the effects on different MRI markers, and between MRI markers and performance gain in working memory (i.e., N-back task).

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<th>Demographic characteristics.</th>
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<td>N (n females)</td>
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<td>APOE e4 [N]</td>
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<tr>
<td>Depression [GDS score]</td>
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<td>CERAD [Total score]</td>
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*Note.* Mean and SD values (except for “N”) are provided. APOE, Apolipoprotein E. GDS, Geriatric depression scale (max. score: 15 with a cut off of 6) \(^{28}\). CERAD, Consortium to Establish a Registry for Alzheimer’s Disease. Total score (max. 100) based on \(^ {29}\) with components from verbal fluency, Boston Naming Test, constructional praxis, word list learning, word list recall, word list recognition.

**White matter microstructure in prefrontal pathways is improved after brain stimulation.** We performed individual probabilistic tractography seeding from the stimulation target (left middle frontal gyrus) using pipelines from FSL \(^ {30}\) to delineate prefrontal white matter pathways. This method repeatedly samples the distribution at each voxel to produce ‘streamlines’ that connect voxels from the selected seed region. To
quantify microstructural integrity in these pathways before and after the intervention, FA values were extracted (averaging individual voxel values along the tract) for both time points and groups and entered into linear model analyses with values post intervention as dependent variables and group as between-subjects factor (including pre intervention FA values, age, and sex as covariates). We observed a group difference with higher FA values along the tract in the anodal compared to the sham group post-intervention ($t_{41} = -2.607$, $p = 0.013$, partial $\eta^2 = 0.14$; model-derived adjusted estimated means [CI]: 0.348 [0.343, 0.354] for anodal and 0.339 [0.334, 0.344] for sham group). Pre FA values were positively associated with higher post FA values ($t_{41} = 10.343$, $p < 0.001$, partial $\eta^2 = 0.72$). No interaction of pre-training FA values with stimulation group was observed and therefore no interaction term was included in the final model. No substantial associations of age and sex to post FA were observed ($t$'s < 1.22, $p$'s > 0.23, partial $\eta^2$'s < 0.05). Tract volumes did not change through the intervention ($t_{41} = 0.547$, $p = 0.587$, partial $\eta^2 = 0.01$; model-derived adjusted estimated means [CI]: 3905 [3669, 4141] for anodal and 3814 [3563, 4065] for sham group). In sum, FA within the structural target network increased more in the training group that had received anodal tDCS compared to sham tDCS, suggesting that active tDCS-plus-training improved white matter tract microstructure.

**Grey matter microstructure is increased after brain stimulation when baseline values are low.** Grey matter regions in the cortex underneath the anode (left middle frontal cortex) were segmented using FreeSurfer 31, overlayed on the stimulation target (Fig. 3) and projected into DTI space to extract individual MD values before and after the intervention. MD values were entered into linear model analyses with values post intervention as dependent variables and group as between-subjects factor (including covariates pre intervention MD, age, and sex). MD values after the intervention were lower in the anodal compared to sham tDCS groups (main effects $t_{41} = -2.30$, $p = 0.027$, partial $\eta^2 = 0.11$) and an interaction of initial MD values by group was found ($t_{41} = 2.29$, $p = 0.027$, partial $\eta^2 = 0.11$). Thus beneficial stimulation effects were larger for individuals with low MD at baseline (e.g., for low baseline values at 25th percentile ($9.9x10^{-3}$), anodal: $9.9x10^{-3}$ [0.8, 1.03x10$^{-3}$], sham: $1.07x10^{-3}$ [1.00, 1.16x10$^{-3}$]; in contrast to those with high baseline values at 75th percentile ($1.15x10^{-3}$), anodal: $1.18x10^{-3}$ [1.13,1.24x10$^{-3}$], sham: $1.13x10^{-3}$ [1.09, 1.18x10$^{-3}$]). Control analyses examined whether macrostructural changes may potentially explain microstructural differences. Therefore, stimulation effects on grey matter volume of middle frontal gyri was evaluated and showed no substantial effect ($t_{42} = 0.110$, $p = 0.913$, partial $\eta^2 < 0.01$, model-derived adjusted estimated means [CI]: 13924 [13764, 14084] for anodal and 13916 [13759, 14073] for sham group). In sum, MD was decreased through training-plus-tDCS in the grey matter underlying the stimulation target, suggesting improved microstructural properties.

**Functional connectivity is increased after brain stimulation.** To investigate whether functional connectivity was modulated by anodal tDCS, we performed seed-to-voxel correlational analyses on resting-state fMRI data using CONN 32. The seed was selected to represent the area under the anode (left middle frontal gyrus from the Harvard-Oxford atlas) and Pearson’s r correlation of the BOLD time course of this seed was computed across the entire brain. Subsequent second-level general linear model
analysis for the group (anodal, sham) × time contrast (pre, post) revealed a significant cluster in the right prefrontal cortex (MNI coordinates: x = 18, y = 18, z = 60, |T_{43}| > 3.53, k = 116, p < 0.05 cluster-size FDR corrected p, voxel threshold: p < 0.001 p-uncorrected, adjusted for the covariates age and sex) in the right superior frontal gyrus (Fig. 4). A more liberal uncorrected p-threshold further supported that the cluster (covering in the right superior and middle frontal gyri) most likely reflects connectivity within the frontoparietal executive control network (MNI coordinates: x = 18/32, y = 18/-4, z = 60/44, |T_{43}| > 2.96, k = 705/145, p < 0.05 cluster-size p-FDR corrected, voxel threshold: p < 0.005 p-uncorrected, adjusted for the covariates age and sex). In sum, FC in the frontal-parietal network increased after training-plus-tDCS, suggesting enhanced network synchronization.

**Pathways’ microstructural plasticity is associated with individual behavioral memory benefit.** In order to examine linear relationships between the effects on different MR markers as well as with performance gain (N-back change), correlation matrices were generated, illustrating scatterplots and Spearman correlation coefficients for all bivariate associations (Fig. 5). We observed a positive association between FA change and N-back change, reflecting that individuals with higher increases in FA also showed more pronounced performance gains ($r_S = 0.402$, $p = 0.006$, anodal: $r_S = 0.436$, $p = 0.23$, sham: $r_S = 0.251$, $p = 0.23$). Neither MD nor FC change showed an association with N-back change ($|r|$’s < 0.299, $p$’s < 0.15). Bivariate scatterplots also revealed that microstructural plasticity in the stimulation target was associated with functional connectivity modulation: Higher decreases in grey matter MD were associated with increases in FC due to the intervention ($r_S = -0.336$, $p = 0.022$) with this relationship being more pronounced in the sham group ($r_S = -0.589$, $p = 0.002$) than in the anodal group ($r_S = -0.009$, $p = 0.97$), indicating that individuals with decreased MD showed increased prefrontal FC due to training. As a control, we explored bivariate monotonic relationships between baseline integrity values (FA in white matter, MD in grey matter, and FC between the target and the resultant cluster) and behavioral performance gain. No substantial associations emerged (Supplementary Fig. 1). Thus, our results suggest a particular association of plasticity in white matter tracts with performance gain of the training-plus-tDCS intervention.

**Discussion**

Three-week brain stimulation-assisted cognitive training in non-demented older adults resulted in modifications of microstructure in white matter pathways and grey matter cortical target area as well as functional connectivity changes in a broader frontoparietal network. FA in prefrontal tracts originating from the stimulation target was increased in the group that had received anodal vs. sham tDCS, indicating higher integrity (i.e., directional preference of diffusion/directional coherence) of frontoparietal white matter tracts which was associated with higher (transfer task) performance gains following the intervention. Further, grey matter microstructure changes differed between the stimulation groups, mainly in individuals with higher microstructural integrity (i.e., molecular diffusion rate/magnitude of water molecule diffusion) at baseline that showed decreased MD values after anodal vs. sham tDCS. Increased resting-state FC between prefrontal areas indicated additional synchronization within frontoparietal
networks induced by tDCS. Overall, we provide evidence for microstructural and network modifications through brain stimulation in the human brain, which may characterize the underlying mechanisms of functional benefits due to the intervention.

We reconstructed individual tracts originating from the stimulation target in the left prefrontal cortex. Fibers connected the prefrontal stimulation target within the fronto-parietal network with ipsilateral parietal as well as contralateral prefrontal areas. FA along these tracts, reflecting microstructural integrity, was increased in the anodal compared to the sham group after the combined intervention.

The DTI-derived index FA reflects the directional preference of diffusion and can be used to quantify the integrity of fiber organization in the human brain with higher values describing higher integrity. Variability between individuals in white matter pathways mediating certain cognitive functions has been shown to predict the variability in behavioral performance. Most intriguingly, these DTI metrics were used to delineate brain plasticity in vivo with their alterations being liked to long-term potentiation (LTP). Thus, cellular modifications through neuromodulatory interventions such as the density, myelin, among others, which are indicative of LTP induction can be studied. Previous studies have observed changes in FA as a consequence of training, that were associated with behavioral performance gain, and even within short periods of time following learning. For example, Hofstetter and colleagues found FA changes in the fornix, induced by a short-term (2 h) spatial training, providing evidence for rapid structural remodeling due to new learning experience. Our previous pilot study examining 3-day spatial training in older adults corroborated these initial results, suggesting that the behavioral relevance of dynamic remodeling in white matter tracts (rather than baseline microstructural integrity per se) is preserved in the aged brain.

In a seminal study investigating structural changes induced by a combined tDCS-and-physical therapy intervention in stroke patients, Zheng and Schlaug observed increased FA in descending motor tracts in the treatment but not in the control group. However, as the control group did not receive any training, the results did not allow conclusions about whether effects were due to tDCS or training or both. Applying anodal tDCS over the left somatosensory cortex during repeated sensory learning, Hirtz and colleagues found FA increases in anodal compared to sham group in the left frontal cortex, in the vicinity of the middle and superior frontal gyrus. The authors concluded that sensory learning involved prefrontal areas rather than stimulation target regions underneath the anodal electrode due to involvement of decision-making processes recruiting the frontoparietal network. In fact, these results together with our complementary findings of tDCS-induced microstructural plasticity in individual tracts may suggest a general (across domains) susceptibility of prefrontal white matter to tDCS-induced neuromodulation.

Candidate cellular mechanisms reflected in FA variations include alterations in cell membrane and fiber density, fiber coherence, axon diameter, myelination, collateral sprouting. Given previous evidence, we believe tDCS may affect the fiber organization and myelin formation through rapid structural remodeling in white matter pathways originating from the stimulation target. These myelination changes affect...
the speed of information processing between brain regions, underlying improvements of performance\textsuperscript{36, 40}. Importantly, the positive relationship of microstructural alterations with behavioral performance gain (as indicated by the transfer N-back task) points towards a functional significance of preserved (brain stimulation-related and learning-related) neuromodulatory plasticity\textsuperscript{5, 7}.

In order to examine microstructural changes within the grey matter of the stimulation target, MD values were extracted. A between-group comparison revealed an interaction between baseline MD values and the stimulation group effect, indicating a decrease after the intervention in the anodal compared to the sham group for individuals with initially lower values in the stimulation target.

The DTI-derived index MD reflects the molecular diffusion rate and is used to quantify tissue microstructure. Higher MD values indicate reduced restriction of water molecule diffusion by cellular structures\textsuperscript{11}. Decreases in MD were also related to brain-derived neurotrophic factor increase (BDNF) which is a marker of LTP\textsuperscript{5}. Next to the expression of BDNF, increases in number of synapses and higher astrocyte activation has been observed and thus discussed as potential underlying mechanisms of learning-induced structural remodeling of neurons and/or glia, sensitive to MD modulation\textsuperscript{3, 5, 6}.

We observed decreased MD after anodal compared to sham group for individuals with initially lower MD in the stimulation target, possibly indicating increases in tissue density (due to reshaping of neuronal or glial processes) or enhanced tissue organization (due to strengthened dendrites or axons) due to tDCS\textsuperscript{5, 41}. Numerically, MD values suggested a slight increase from before to after the intervention, similar to what has been observed after exercise training in older adults: For example, Callow and colleagues found increases in cortical grey matter (insular) MD after training\textsuperscript{42}. These training-induced MD increases were interpreted as reduced inflammation and cellular swelling in the aged brain\textsuperscript{43, 44}. It is also possible that training improved neural efficiency through synaptic and dendritic pruning (reducing density of synapses and dendrites and thus increasing MD values). Together with these findings, our results corroborate the preservation of dynamic properties of glial-related activity for the refinement of synaptic processes in aged individuals. TDCS, however, may also operate upon dendritic spine sprouting and branching and/or synaptogenesis and/or increases of glial cell volume\textsuperscript{3, 14}. In rats, tDCS modulated spinogenesis (increasing the number and affecting the shape of spines) in the auditory cortex, not only inducing the formation of new spines, but also stabilizing already existing connections\textsuperscript{45}. Barbati and colleagues corroborated this finding in motor tDCS-induced spine density modulation in mice which was accompanied by motor skill performance enhancement\textsuperscript{46}. In our data, MD modulation was not related to performance gain, suggesting a more complex relationship. However, MD decreases were related to concomitant functional connectivity modulation through training, a finding that further stresses the impact of structural plasticity on brain network connectivity\textsuperscript{4, 14}.

In order to examine potential functional connectivity modulations, we conducted seed-based FC analyses using resting-state fMRI. We found increases in FC in the prefrontal task-independent frontoparietal network in the anodal compared to the sham group. Similar FC modulations have been observed in task
fMRI during single tDCS applications and after repeated tDCS sessions combined with working memory training in older adults. Nissim and colleagues found state-dependent FC increases due to tDCS-accompanied working memory training, within the targeted frontoparietal network. Enhancement of frontoparietal connectivity has been shown to support working memory processing and capacity. We previously examined the neural effects of repeated combined tDCS-plus-training sessions such as visuospatial memory. Memory network connectivity was shown to be increased in the tDCS compared to the sham group, indicating coherent intranetwork activity to underlie memory function. Corroborating and extending these and previous findings, we here showed that the functional coupling between bilateral prefrontal regions – part of the frontoparietal network – was increased through tDCS, suggesting that enhanced temporal coherence of BOLD activity is one of the mechanisms underlying tDCS effects. By enabling more coordinated/synchronized activity between network hubs, tDCS combined with repeated sessions may produce (potentially longer-term) transfer effects. In our data, we did not observe an association between FC changes and behavioral performance gains, pointing to a rather complex relationship which may also be masked by the impact of baseline FC on behavioral modulation. This also highlights the usefulness of multimodal imaging, including comprehensive examination of both grey and white matter plasticity, to uncover the relationships of different levels of effects.

In sum, the present study advances the understanding of neurobiological after-effects of non-invasive brain stimulation combined with repeated training interventions and shows that tDCS exerts its effects on multiple levels, including microstructural properties of white matter tracts and grey matter regions and coordinated activity between distant brain regions. This rapid remodeling of neuro-glial networks and long-range signaling as the result of neuromodulation may underlie the functional effects, as indicated by their (partial) association with the observed performance gains. Our findings encourage future studies to assess the dynamic properties of microstructural alterations in the human brain in more detail, administering DTI scans within shorter time frames with regard to tDCS-assisted learning. In addition to time scale of remodeling, regional differences remain to be explored in future studies, determining whether neuromodulation exerts similar modulation when applied to other networks. Moreover, it is unclear if findings from the present cohort extend to other (patient) samples as neuromodulatory plasticity may differ as function of brain changes in different diseases. Insights from future investigations will further increase knowledge at microstructural and brain network levels and determinants of responsiveness to stimulation. In a subsequent step, this knowledge may help to develop longer-lasting effects, and potentially to individualize stimulation parameters including optimal positioning of electrodes and stimulation intensity, in order to maximize functional benefits in experimental and clinical applications.

**Methods**

**Participants.** Data of 48 nondemented older adults was used for the present study (see Table 1). All participants were right-handed, native German speakers, had no history of neurological or severe psychiatric diseases, did not take any central nervous system active medication, and performed within...
age- and education-adjusted normative range in the neuropsychological screening (Consortium to Establish a Registry for Alzheimer's Disease, CERAD-Plus Test Battery, https://www.memoryclinic.ch). All participants completed the TrainStim-Cog clinical study where they received anodal or sham transcranial direct current stimulation over the left prefrontal cortex during three weeks (totaling up to nine sessions) of a training of two executive functions tasks (a letter updating task and a value-based Markov decision making task \(25,26\), NCT03838211, https://clinicaltrials.gov/show/NCT03838211). All behavioral data is reported in \(25\). In the present study, we analysed the magnetic resonance (MR) imaging data acquired in 48 participants – including resting-state functional MR imaging, structural T1 imaging, and diffusion tensor imaging (DTI) – before and immediately after the three-week intervention. The study flow chart is shown in Fig. 1. The study was approved by the ethics committee of the University Medicine Greifswald and conducted in accordance with the Helsinki Declaration. All participants gave written informed consent before participation.

**Cognitive training with concurrent anodal tDCS over the left prefrontal cortex.** Cognitive training consisted of a letter updating task \(^5_3\) and a three-stage Markov decision-making task \(^5_4\). In the letter updating task, different lists of letters A to D with varying length were presented in random order. After each list, participants were asked to recall the last four letters presented. For the Markov decision-making 3D characters were presented, prompting participants to choose between two actions, which resulted in an action-related outcome (either in terms of monetary gain or loss). Hence, participants had to learn to choose the optimal sequence of actions to maximize their overall gains and minimize overall losses. A numerical n-back-task comprised of a 1-back and a 2-back condition, was used as to assess transfer to an untrained working memory task. Each condition consisted of nine trials and 10 items. The task was applied at the session before training (pre) and after training (post). All details including other cognitive tasks are described in \(^25,26\). Cognitive training was accompanied with either anodal or sham tDCS via a battery-operated stimulator (Neuroelectrics Starstim-Home Research Kit). Two circular saline-soaked sponge electrodes (5-cm diameter; anode: F3, cathode: Fp2) mounted in a neoprene head cap were applied using the 10–20 EEG-system grid. Direct current was delivered with 1 mA intensity for 20 minutes in the anodal tDCS group and for 30 seconds in the sham group. The stimulation was started simultaneously with the letter updating task (and finished after approximately the first half of the Markov task).

**MRI data acquisition.** MR images were acquired at the Baltic Imaging Center (Center for Diagnostic Radiology and Neuroradiology, University Medicine Greifswald) on a 3-T Siemens verio scanner using a 32-channel head coil. Resting-state fMRI scans were acquired using an echo-planar-imaging sequence (3 x 3 x 3 mm\(^3\) voxel size, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle = 90 °, 34 slices, descending acquisition, field of view 192 x 192 mm\(^2\), 176 volumes, TA = 6.00 min). Participants were instructed to keep their eyes closed, to not think of anything in particular, and to try not to fall asleep. High-resolution anatomical images were acquired using a three-dimensional T1-weighted magnetization prepared rapid gradient echo imaging (1 mm\(^3\) isotropic voxel, TR = 2300 ms, TE = 2.96 ms, inversion time = 900 ms, flip angle = 9°, 256 x 240 x 192 mm\(^3\) matrix). Further, a diffusion-weighted spin-echo echo-
planar imaging sequence was acquired (1.8 x 1.8 x 2.0 mm³ voxel size, TR = 11100 ms, TE = 107 ms, 70 slices, 64 directions (b = 1000 s/mm²), 1 b0).

**MRI data analyses.** *Structural T1-weighted images and DTI analysis.* T1 and DTI data were processed by Freesurfer version 6 (https://surfer.nmr.mgh.harvard.edu) and FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). First, T1 data were processed by the FreeSurfer’s cross-sectional pipeline (recon-all) which includes motion correction, skull stripping, normalization, intensity correction, volumetric segmentation, and cortical surface reconstruction. Second, the longitudinal pipeline was applied in order to create a robust, unbiased which-subject template using robust, inverse consistent registration which increases reliability and statistical power, for the detection of brain structural changes that may occur with intervention. Quality assessment involved visual inspection of all processing steps and calculation of anatomical signal to noise ratios using Freesurfer QAtools (https://surfer.nmr.mgh.harvard.edu/fswiki/QATools). All structural data were deemed appropriate for analysis.

Regional volumes were extracted for the ROI corresponding to the stimulation target (i.e., left middle frontal gyri from the Desikan-Killiani atlas) and adjusted for total intracranial volume (ICV) using the residual-method.

DTI data preprocessing included eddy current and head motion correction using an automated affine registration algorithm. A diffusion tensor model was fitted to the motion-corrected DTI data at each voxel to create individual 3-dimensional FA and MD maps. FSL’s BEDPOSTX was used to calculate the distribution of fiber orientations at each brain voxel. We used a seed-based probabilistic approach to track prefrontal white matter fibers.

Probabilistic fiber tracking was conducted with PROBTRACKX2 implemented in FSL; this method repeatedly samples the distribution at each voxel to produce ‘streamlines’ that connect voxels from selected seed regions. The following parameters were applied: 5000 streamline samples, 0.5 mm step length, curvature threshold = 0.2. The left middle frontal gyrus from the Harvard-Oxford atlas also used for resting-state fMRI analyses (see below), transformed into individual DTI space, multiplied with diffusion maps and binarized, was used as seed regions for the tracts. Given the large size and extent of prefrontal streamlines, these paths were thresholded by 10% of the individual tract-specific connection probability to reduce the likelihood of including extraneous tracts. A canonical image of the thresholded probabilistic tract is provided in Fig. 2. To generate the canonical image, individual tracts from all participants were normalized, converted to binary images and then summed (color coding reflects the probability of voxel to be present in 33–100% of the participants). All data were visually inspected for major artifacts before being included in analyses. Fractional anisotropy (FA) was used as our measure of tract integrity, given that earlier studies have indicated it to be a reliable assessment of microstructural integrity of white matter fibers. Mean FA for all streamlines was then calculated by
masking the tracts with individual diffusion maps, binarizing to define tract masks, and averaging individual voxel values along the tract which was then entered into statistical analyses.

Individual T1-weighted images were coregistered to the b0 images, using rigid-body transformation. These registrations were used to transform masks of the left stimulation target to the MD maps. To extract MD from the grey matter within the stimulation target, the individually segmented left middle frontal gyrus was masked by the ROI used for seed-based tractography and rsFC analyses, in line with previous studies \(^{58, 63}\).

**Resting-state FC analysis.** Resting-state fMRI data was analysed using CONN toolbox version 21 (www.nitrc.org/projects/conn) \(^{32}\). Data preprocessing consisted of functional realignment, slice-time correction, structural segmentation and normalization to the Montreal Neurological Institute (MNI) template, functional segmentation and normalization, and smoothing using a 6-mm Gaussian kernel. Denoising of the blood oxygenation level-dependent (BOLD) signal from physiological and other sources of noise was performed using the CompCor method, implemented in the toolbox \(^{64}\). The residual BOLD time series were then high pass filtered at 0.01 Hz. Intermediate motion thresholds (0.9 mm slice-to-slice movement and global mean signal below 5 SD) were chosen. Mean and maximum framewise displacement as motion quantities in the anodal and sham group are displayed in Table 2. Scrubbing was implemented as part of the CONN preprocessing pipeline through the Artifact detection toolbox (ART, http://www.nitrc.org/projects/artifactDetect/) by regressing noise components for outlier scans from the BOLD signal as part of denoising \(^{65}\). Data with number of detected outlier scans exceeding 3 SD of the sample mean number of outlier scans (mean 7, SD 22) were excluded from further resting-state analyses, resulting in exclusion of one participant with 147 detected outlier scans (corresponding to 42% of acquired volumes). All segmentation, normalization and registration steps were visually inspected and were deemed appropriate for analysis.

After preprocessing and denoising, first-level (within-subjects) connectivity maps for each participant were entered into whole-brain region analyses. Second-level (between-subjects) general linear analyses were modeled with a 2 (groups: anodal, sham) x 2 (time points: pre, post) design. The interaction between group and time point was assessed to examine whether functional connectivity alterations from pre to post differed between anodal and sham groups. Age and sex were included as covariates. Analyses were corrected for multiple comparisons using a false discovery rate (FDR)-corrected p-value of 0.05 at cluster-level (height threshold of uncorrected p < 0.001).
Table 2
Motion parameters of DTI, rsfMRI data, and T1.

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<th>total</th>
<th>anodal</th>
<th>sham</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DTI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>translation (mm)</td>
<td>1.2 (0.11)</td>
<td>1.2 (0.10)</td>
<td>1.2 (0.11)</td>
</tr>
<tr>
<td>rotation (degrees)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>post</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>translation (mm)</td>
<td>1.2 (0.11)</td>
<td>1.2 (0.10)</td>
<td>1.2 (0.12)</td>
</tr>
<tr>
<td>rotation (degrees)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>rsfMRI</strong></td>
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<td></td>
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<tr>
<td>mean FD (mm)</td>
<td>1.0 (0.6)</td>
<td>1.0 (0.5)</td>
<td>1.0 (0.6)</td>
</tr>
<tr>
<td>max FD (mm)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td><strong>T1</strong></td>
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<td></td>
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</tr>
<tr>
<td>pre: anatomical SNR</td>
<td>20.6 (2.9)</td>
<td>20.7 (2.7)</td>
<td>20.6 (3.2)</td>
</tr>
<tr>
<td>post: anatomical SNR</td>
<td>20.4 (3.0)</td>
<td>20.7 (2.6)</td>
<td>20.2 (3.3)</td>
</tr>
</tbody>
</table>

*Note.* Mean and SD values are provided. rsfMRI, resting-state functional magnetic resonance imaging. FD, framewise displacement. DTI, diffusion tensor imaging. SNR, signal-to-noise ratio.

**Statistical analyses.** To assess the statistical significance of differences in microstructural MRI markers between stimulation conditions, R\(^66\) was used including the packages emmeans\(^67\), tidyverse\(^68\), ggplot2 and GGally\(^69\). Linear models were calculated for each dependent variable (FA/MD after intervention). Models were adjusted for age, sex, and respective baseline value. Model-based post-hoc comparisons of estimated fixed effects were computed. T-values, degrees of freedom and p values are reported in the results section. A two-sided significance level of \(\alpha = 0.05\) was used. No multiple comparison adjustment for p-value was performed.

**Declarations**

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**Author contributions**

D.A. and A.F. conceived the study and designed the experiments; F.T. performed the experiments and collected the MR data; D.A. supervised data collection; D.A., F.T. and A.E.F. processed the MR data; D.A. and A.E.F. analyzed the data and prepared the figures; D.A. and U.G. performed statistical data analysis; D.A., M.M., and A.F. interpreted the results and wrote the paper; all authors reviewed the paper.

** Competing interests**

The authors declare no competing interests.

**Data availability**

The data of this study are available upon reasonable request from the corresponding author D.A. The data are not publicly available due to potential identifying information that could compromise participant privacy.

**References**


Study flow chart. Following a Pre-assessment of performance on the cognitive tasks, a pre-intervention MRI was conducted; the intervention commenced two days later and lasted for three weeks (with active (anodal) or sham tDCS + training administered three times per week). A post-intervention MRI session was conducted two days after the end of the intervention period. MRI = magnetic resonance imaging. tDCS = transcranial direct current stimulation. 'Exclusion of n=2 (one from anodal in post, one from sham group in pre) in tractography analysis due to missing DTI data. *Exclusion of n=1 from sham group resting-state fMRI analysis due to excessive motion during the functional scan. The tDCS montage targeted the left middle frontal gyrus as major node of the frontoparietal network, therefore, the anode was placed over F3 with the cathode over the contralateral supraorbital site (intensity: 1 mA, duration: 20 min in anodal, 30 sec in sham group). tDCS started concurrently with the cognitive training.
**Figure 2**

**White matter pathways’ microstructure (fractional anisotropy, FA).** A canonical image of the thresholded probabilistic tract, overlaid on the Montreal Neurological Institute (MNI) brain, is provided on the left. To generate the canonical image, individual tracts from all participants were normalized, converted to binary images and then summed (color coding reflects the probability of voxels to be present in 33% to 100% of the participants). FA along the tracts was increased after training in the training group that had received anodal compared to sham tDCS.
Figure 3

**Grey matter microstructure in the stimulation target (mean diffusivity, MD).** The left middle frontal gyrus (IMFG), selected as the (grey matter) stimulation target, overlaid on the MNI brain, is provided on the left. MD values were decreased after the intervention in anodal compared to sham group for those individuals with initially lower MD in the stimulation target.
Seed-based functional connectivity (FC) with seed in stimulation (left middle frontal gyrus) to the rest of all voxels in the brain. Seed-based FC analyses showed a significant cluster (p FDR 0.05, p unc 0.001) in the right superior and middle frontal gyri, reflecting an increase of FC to the stimulation target after the intervention in anodal compared to sham group.
Figure 5

Scatterplots for correlations between Post-Pre differences in FA, MD, and FC with individual performance gain (N-back change). Increased FA change was associated with increased behavioral performance gain. Decreased MD changes were associated with FC increases. FA, fractional anisotropy. MD, mean diffusivity. FC, functional connectivity. Blue bars/points/0: sham group. Orange bars/points/1: anodal tDCS group. \( p < 0.10 \) * \( p < 0.05 \) ** \( p < 0.01 \).
Supplementary Files

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